

## EXHIBIT B, PAGE 1

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→ Sacrifice of mice from experiment: adjuvant effect of chemokines (hMCP-4, hMIP-3 $\alpha$ , hTECK Peprotech) on the anti- $\beta$ gal response (plasmid).

Immunizations: injection of 50  $\mu$ l PBS or 50  $\mu$ l chemokine diluted in PBS (= 100 ng), and 3 h after footpad injection of 50  $\mu$ g (50  $\mu$ l) pLacz or pcDNA3, very low endotoxin (<5 EU).

Same injections ate Day 0,7,14,21. Blood sampling for serum at Day 0,14,28.

Day 28: sacrifice of all mice (groups of 6 female BALB/c mice, 6-week old at the beginning of the protocole). Recovery of spleen and popliteal lymph nodes. Single suspension and red blood-cell depletion for spleen.

Cell counts

	Popliteal lymph node		spleen	
	Total x 10 <sup>6</sup>	/animal x 10 <sup>6</sup>	Total x 10 <sup>6</sup>	/animal x 10 <sup>6</sup>
PcDNA3	9.66	1.61	265	44.17
PBS + pLacz	18.96	3.16	285	47.50
MIP-3 $\alpha$ + pLacz	21.6	3.6	250	41.67
MCP-4 + pLacz	33	5.50	325	54.17
TECK + pLacz	22.8	3.8	300	50.00

Culture set up in clone medium + 20 ng IL-2 + 1  $\mu$ M BP-1 with /ml: 5 x 10<sup>6</sup> spleen cells (24 well/group) or 2 x 10<sup>6</sup> lymph node cells (all cells put in culture).

Day 3: add 1 ml medium + IL-2 + BP-1

*Page 165:*Friday

*Experiment irrelevant here*

Monday

*First experiment irrelevant here*

→ ELISA for the adjuvant effect of hMCP-4, hMIP-3 $\alpha$  (not dosed for TECK on that day because not enough coated plates) cf p164 for groups.

Standard protocole for the dosage of IgG, IgG1, IgG2a

- coat 5  $\mu$ g/ml, 50  $\mu$ l in PBS o/n @ 4°C
- 2 washes PBS
- block 2h @ 37°C
- pooled samples, 1/40 dilution than 1/2 to 1/2 dilutions, triplicates, 1 h @ RT
- anti Igs 1/1000 SAV-PAL 1h
- substrate 30 min
- OD 405-490.

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*Raw data tables Day 0 and Day 14*

A:pcDNA3            C: hMIP-3 $\alpha$  + pLacz  
B: pLacz (+PBS)    D:hMCP-4 + pLacz  
A1,2,3: dilutions 1/40, 1/80, 1/160 ...

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*Raw data tables Day 28*

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*Final table*